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Characteristics and outcomes of patients with formiminoglutamic aciduria detected through newborn screening

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Summary

Background—Glutamate formiminotransferase deficiency (FTCD deficiency) or formiminoglutamic aciduria is the second most common of the known inherited disorders of folate metabolism. Initial case reports suggested that patients may have severe intellectual disability and megaloblastic anemia. However, these cases were obtained from screening cohorts of patients with developmental delay. Subsequently, patients with milder clinical phenotypes have been reported. The full phenotypic spectrum of this disorder remains unknown.

Methods—In many states, FTCD deficiency can be incidentally detected on tandem mass spectrometrybased newborn screening of dried blood spots. In this work, we report the outcomes of infants identified to have FTCD deficiency through newborn screening.

Results—During the study period, 18 patients were identified to have FTCD deficiency and were referred and evaluated at one of the two participating metabolic centers. The overall rate of FTCD deficiency detected through the New Jersey screening program over the study time period was

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Contributions:

RA: project design, data collection, data analysis, drafting of manuscript, editing of manuscript
RG: project design, data collection, data analysis, drafting of manuscript, editing of manuscript
PR: project design, data collection, data analysis, editing of manuscript
RC: project design, data collection, data analysis, editing of manuscript
CF: project design, data collection, data analysis, editing of manuscript

Conflicts of Interest:

RA: No conflicts of interest to disclose
RG: Received a one-time consulting fee from Dudnyk Healthcare Marketing
PR: No conflicts of interest to disclose
RC: Site PI for industry-sponsored clinical trial (BioMarin Pharmaceutical Inc). Past site PI and *ad hoc* consultant for Horizon Therapeutics, Inc.
CF: Provided consulting support to and received grant support and honoraria for speaking engagements from BioMarin Pharmaceutical Inc., Abbott Laboratories, Alexion Pharmaceuticals Inc., Shire, Pfizer, Swedish Orphan Biovitrum (Sobi), Sanofi-Genzyme, and Horizon Therapeutics, Inc.

Ethics approval: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). The study was approved by the IRB of both the Children's Hospital of Philadelphia and the Children's Hospital of Michigan, and because there was no more than a minimal risk to subjects, informed consent to review the charts was waived.

1:58,982. At a mean age of 56 months at last follow-up, 3/18 (16%) had developmental delays requiring individualized education plans; no patients had profound intellectual disability. 4/16 (25%) had mild self-limited anemia; no patients had profound anemia.

Conclusions—These data suggest that the majority of individuals with FTCD deficiency detected by newborn screening are asymptomatic.

Keywords

Glutamate formiminotransferase deficiency; formiminoglutamic aciduria; newborn screening; intellectual disability; anemia

Introduction

Formiminoglutamic aciduria (FIGLU-uria or FTCD deficiency) is one of five known inborn errors of folate metabolism. It is the second most common folate disorder, with a reported incidence of approximately 1:46,000 (Majumdar et al. 2017). The deficiency is due to mutations in the *FTCD*-encoded bifunctional protein, which is required for histidine metabolism. The protein product serves two enzymatic functions, first the formiminotransferase domain catalyzes the conversion of formiminoglutamate (FIGLU) to 5-formiminotetrahydrofolate. In the subsequent step of the pathway the cyclodeaminase domain catalyzes the cyclodeamination of the formimino group yielding 5,10-methenyltetrahydrofolate and ammonia (Hilton et al. 2003).

Patients have a characteristic elevated excretion of FIGLU in the urine. The clinical features of FTCD deficiency have been reported in less than 15 individuals. In these limited cases, there appears to be a wide variety of clinical phenotypes. Early publications suggested a severe intellectual disability and megaloblastic anemia phenotype in a group of Japanese patients (Arakawa et al. 1965; Arakawa et al. 1968). The elevated FIGLU levels in these severe patients occurred only in response to a histidine load, with a normal FIGLU level at baseline. Of note, these individuals were ascertained through evaluation of their known intellectual disability.

Subsequent patients with no hematologic abnormalities and possible mild developmental delay have been described (Niederwieser et al. 1974; Perry et al. 1975; Beck et al. 1981; van Gennip et al. 1994). Patients with the mild clinical phenotype appear to have more profound biochemical abnormalities with elevations in urine FIGLU levels even in the absence of a histidine load. It has been proposed that mutations associated with severe disease result in decreased activity in the cyclodeaminase domain, while mild phenotypes result from reduced activity of the formiminotransferase domain (Rowe 1983); however, a biochemical analysis of three patients suggests this may not be the case (Hilton et al. 2003).

A recent publication detailed the allelic spectrum FTCD deficiency in 20 individuals with biochemically-confirmed disease (Majumdar et al. 2017). Twelve distinct pathogenic variants were identified, including five missense changes, one inframe deletion, four nonsense variants, and two frameshift alterations resulting in premature termination codons. These changes spanned both major functional domains of the protein. Most of their cases

were ascertained through the Michigan newborn screening program. While this work provides important information about the molecular basis of FTCD deficiency, it did not report the subjects' clinical phenotypes.

Infants with FTCD deficiency have been incidentally detected through tandem mass spectrometry-based newborn screening assays designed to identify elevations in C4acylcarnitine as a marker of short chain acyl-CoA dehydrogenase deficiency (SCAD) or isobutyryl-CoA dehydrogenase deficiency (IBD). On MS/MS analysis of butylated acylcarnitine derivatives, elevated C4-acylcarnitine is seen as an increased 288.2 *m/z* ion signal, while FIGLU results in an increase of a 287.2 *m/z* ion (Malvagia et al. 2006). The first ^{13}C isoptomer of FIGLU, however, produces a 288.2 *m/z* species, which can be mistaken for the C4-acylcarnitine. The species can be differentiated by the presence of a signal at 287.2 *m/z*, the identification of which denotes FIGLU. While NBS provides an unbiased way to screen for significant elevations in FIGLU, it is not clear if the original FTCD patients that only show FIGLU elevations in response to a histidine load would have been identified through NBS.

During the study period of this work, both the newborn screening programs of Michigan and New Jersey referred cases with elevated FIGLU for further metabolic evaluation and follow-up. This allowed for identification of a cohort of FTCD deficiency patients. We report the outcomes of these individuals here.

Methods

This is a retrospective analysis of infants identified to have FIGLU on newborn screening. Data were combined from two study sites: 1) The Children's Hospital of Michigan Metabolic Clinic, a referral site for the newborn screening program of Michigan, and 2) The Children's Hospital of Philadelphia, a referral site for the newborn screening programs of New Jersey and Pennsylvania.

Inclusion criteria for the study included 1) birth date between January 1st 2006 and December 31st 2015; 2) the presence of elevated C4 or FIGLU level on NBS; 3) referral to one of the two study sites; 4) biochemical or genetic confirmation of FIGLUuria. Subjects were excluded if they were found to have a false-positive NBS, or if they were referred but never actually seen in one of the two participating metabolic clinics.

From 2006-2011, the Michigan NBS program used a butylated acylcarnitine derivative MS/MS technique for acylcarnitine analysis, and therefore identified FTCD deficiency cases through identification of a large C4 peak. In 2011, the Michigan program changed methods to a non-derivatized method that allowed for discrimination between the C4 and formiminoglutamate peaks. At this time, the decision was made to no longer report out abnormal FIGLU newborn screens. The New Jersey NBS program reported elevated FIGLU cases from 2009-2015.

Outcomes data were collected by review of the documentation from initial and follow-up visits in the electronic medical record, paper chart, as well as clinical communication received from the primary care provider.

Results

Between 2009-2015 there were 707,785 births in the state of New Jersey, and 12 confirmed FTCD deficiency cases for an overall incidence of 1 in 58,982. This is comparable with the previously reported rate of 1 in 46,000 among infants born in Michigan (Majumdar et al. 2017). Among the 12 FTCD deficiency patients born in New Jersey, 5 were evaluated at the Children's Hospital of Philadelphia (CHOP) and were therefore included in this analysis. The remaining 7 were referred to other centers. One additional patient was referred to CHOP by the Pennsylvania NBS program and was included in the study.

During the study period, 15 patients were referred to the Children's Hospital of Michigan. Three were excluded from analysis because they were never evaluated after the referral. One of those excluded died of complications of extreme prematurity in the setting of a triplet gestation. Another died of complications of prematurity and presumed sepsis. The third patient excluded from analysis sought metabolic care at a center in a different state.

The demographics and birth information for the 18 subjects evaluated and followed at a study site are shown in Table 1. One of the Michigan patients (Patient 14) was identified after routine screening for FIGLU stopped in Michigan because of a sibling with a history of an abnormal NBS. The population included an equal number of males and females, with a mean gestational age of 37.7 weeks. No infants demonstrated symptoms of metabolic decompensation. Most had uncomplicated neonatal courses with the exception of two infants who required admission to a neonatal intensive care unit for prematurity, and three additional infants who required phototherapy for hyperbilirubinemia. One infant was also diagnosed with an atrial septal defect.

Newborn screening and confirmatory biochemical testing results are shown in Table 2. Only 28% (5/18) of newborn screen results were explicitly reported as elevated FIGLU, the remainder were reported as increased C4-acylcarnitine. All infants had elevated FIGLU on plasma acylcarnitine profile, with a mean value of 1.9 μmol/L (for results that were explicitly listed). FIGLU levels were also elevated in urine with a mean value of 108 mg/g creatinine (for results that were explicitly listed). Patients seen at the Pennsylvania site also had measurement of hydantoin-5-propionic acid, a by-product of the histidine metabolism pathway that accumulates in patients with FTCD deficiency (Niederwieser et al. 1976), with a mean value of 86.5 mg/g Cr. Folate levels were also elevated in most subjects (13/15); exact numbers varied greatly due to inter-laboratory variability in reference ranges.

FTCD genotype and outcomes data are presented in Table 3. A novel *FTCD* variant of uncertain significance (c.236A>C, p.Q79P) was identified in a biochemicallyconfirmed patient (subject 4) that also carried the previously-reported c.1366dupG (p.E456Gfs*56) mutation. All other alleles in our population are previously reported as known pathogenic changes. Pathogenic mutations disrupting the foriminotransferase (p.Q79P, p.R255X, p.M75L, p.G172W, p.K151X, p.C107R), linker (p.P331Afs*2) and cyclodeaminanse domains (p.E456Gfs*56, p.L536X) were identified. This suggests that mutations throughout the protein can lead to biochemical abnormalities that are detectable through NBS.

Follow-up evaluation through review of the medical record revealed that 25% (4/16) had mild self-limited anemia. 3/18 children had developmental delays requiring educational accommodation. One child was diagnosed with autism, while two additional children required educational support for cognitive delays. Two additional children had mild speech delays. All of these children are verbal and are making developmental progress. No children were identified to have severe intellectual disability.

Discussion

Glutamate formiminotransferase deficiency is the second most common of the known inherited disorders of folate metabolism, with several cases known from published case reports (Hilton et al. 2003). Despite this relative frequency, the phenotypic spectrum has not been well-characterized. This report doubles the number of described cases (Table 4).

In many states, FTCD deficiency is an incidental finding on newborn screening because the C-13 isotopomer of the butyl-ester of FIGLU has a mass-to-charge ratio of 288.2 *m/z*, identical to that of the butyl-ester of C4-carnitine, causing false elevations of C4-carnitine. However, the primary isotopmer of FIGLU butyl-ester is at a mass to charge ratio of 287.2 *m/z*, a very distinctive finding on acylcarnitines, so the conditions can be distinguished by careful analysis(Malvagia et al. 2006). Our cohort is composed of patients who were detected on newborn screening, which is unique as compared to previously reported patients, who were ascertained on metabolic screening obtained due to symptomatic manifestations.

In our cohort of FTCD deficiency patients identified through NBS, 72% (13 of 18) had normal development and 75% had no history of anemia. This supports the hypothesis first put forward by Niederwieser et al.(Niederwieser et al. 1974) that a benign form of FTCD deficiency exists. It is difficult to know if the one case of autism and two additional cases of developmental delay requiring educational accommodation can be attributed to the FTCD deficiency. One of these patients was the product of a first degree consanguineous mating, and therefore is at increased risk of other inherited causes of intellectual disability. In terms of the anemia, while four patients had mild anemia, there were no cases of the profound anemia that was reported in early publications. The mutations found in patients with anemia or developmental delays did not cluster in one functional domain of the FTCD protein (further supporting the hypothesis of Hilton et al. 2003).

We suggest that based on these findings there is no apparent systemic association of NBS-identified FTCD deficiency with severe intellectual disability. Individuals identified through NBS should undergo typical developmental surveillance as part of their general pediatrics care. If any delays are identified appropriate referrals to early developmental therapies should be initiated, as would be in any other child.

Of note, many states are removing C4 as a primary NBS marker, as they are eliminating Short-chain Acyl-CoA Dehydrogenase Deficiency (SCAD) and IsobutyrylCoA Dehydrogenase Deficiency (IBD) screening. This will eliminate FTCD deficiency screening as well. Furthermore, since FTCD deficiency is not on the Recommend Universal Screening

Panel, several states, such as Michigan, which can biochemically differentiate between C4 and formiminoglutamate are choosing not to report it.

The question remains as to how to manage incidental findings of mutations in *FTCD*, a question that we anticipate will become increasingly common in this era of next-generation sequencing. Indeed, mutations in *FTCD* are being discovered and reported as causative findings on whole-exome sequencing (Yang et al. 2014). It has been previously speculated that there exist two populations of FTCD deficiency patients: high-excretors, with relatively benign features and low-excretors with severe intellectual disability (Hilton et al. 2003). In some low-excretors, FIGLU was only elevated after histidine loading. Therefore, it is possible that there still exists a severe subpopulation that has such low basal levels of the butyl-ester of FIGLU that they are not detectable on newborn screening. To fully understand the significance of an FTCD variant, one may need to consider measuring a response to a histidine load. Careful biochemical phenotyping of patients with exome-identified variants in the *FTCD* gene in conjunction with long-term follow-up of individuals identified on NBS are needed to fully define the clinical spectrum of *FTCD* deficiency.

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Synopsis

Glutamate formiminotransferase deficiency has been reported to be associated with severe anemia and intellectual disability; through unbiased population-wide newborn screening, we have identified that a large percentage of patients with this biochemical abnormally are healthy, with normal development and hematologic evaluation.

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Table 1

Patient Characteristics

Subject	Study Site	Sex	Pregnancy Complications	Gestational Age (wks)	Neonatal Course
1	PA	F	none	39	normal
2	PA	F	twin	38	normal
3	PA	F	none	36	normal
4	PA	F	none	39	normal
5	PA	F	none	40	normal
6	PA	M	gestational DM	35	NICU stay for respiratory distress
7	MI	M	gestational DM preeclampsia non-reassuring fetal heart tones	33	NICU stay for respiratory and feeding support, hyperbilirubinemia
8	MI	M	genital herpes	40	hyperbilirubinemia
9	MI	F	hyperemesis	39	atrial septal defect diagnosed
10	MI	M	non-reassuring fetal heart tones	37	hyperbilirubinemia
11	MI	M	none	38	normal
12	MI	M	none	39	normal
13	MI	F	none	40	normal
14	MI	M	twin	38	normal
15	MI	F	none	37	normal
16	MI	F	decreased amniotic fluid breech presentation	37	hyperbilirubinemia
17	MI	M	gestational DM, maternal use of clonopine and zyprexa, consanguinity (parents are 1st degree relatives)	36	poor feeding, readmission at 12 days for hyponatremic dehydration in setting of free water intake
18	MI	M	preeclampsia	39	normal

Table 2

Biochemical findings in neonates

Subiect (state)	NBS C4 in µmol/L (PA norm <1.0 Ml norm <1.64)	FlGLU explicitly noted on NBS?	Artificial elevation of confirmatory C4 in µmol/L on plasma acylcarnitine profile (norm <1)	Confirmatory FlGLU in µmol/L on plasma acylcarnitine profile (norm =0)	Confirmatory plasma amino acids in µmol/L	Confirmatory urine FlGLU in (norm=undetectable)	Confirmatory hvdantoin-5-propionic acid on urine organic acids in mg/g Cr	Confirmatory relate level elevated?
1 (PA)	2.42		1.41	increased	methionine 17.7 (16-43) repeat 26. &	not done	not done	+
2 (PA)	2.23		0.61	increased	intermittent elevation in glutamine	not done	153	+
3 (PA)	reported as elevated FlGLU	+	1.72	increased	no significant abnormalities	not done	65	+
4 (PA)	0.0		2.27	increased	not done	not done	not done	not done
5 (PA)	1.14		1.7	increased	not done	not done	28	not done
6 (PA)	1.86		1.16	increased	no significant abnormalities	not done	100	+
7 (Ml)	2.75		3.6	7.69	not done	134.6	not done	+
8 (MI)	2.63		normal	1.06	not done	16.6	not done	+
9 (Ml)	1.69		0.87	1.2	not done	164.9	not done	+
10 (Ml)	2.48		0.75	0.47	mild tyrosine elevation that improved	167.5	not done	+
11 (Ml)	1.68		normal	0.55	not done	63.3	not done	+
12 (Ml)	3.07		normal	increased	no significant abnormalities	259.4	not done	−
13(Ml)	1.44 (nl <0.71 detected on 2nd NBS sent for thyroid dysfunction)		normal	0.9	not done	69.1	not dene	+
14 (Ml)	referred because of sib with FlGLU, after Ml discontinued screening		normal	increased	not done	80.6	not done	+
15 (Ml)	2.06	+	normal	increased	histidine 162 (nl 40-120)	90.7	not dene	+
16 (Ml)	1.73	+	normal	1.12	not done	28.0	not done	+

Subject (state)	NBS C4 in µmol/L (PA norm <1.0 MI norm <1.64)	FIGLU explicitly noted on NBS?	Artificial elevation of confirmatory C4 in µmol/L on plasma acylcarnitine profile (norm <1)	Confirmatory FIGLU in µmol/L on plasma acylcarnitine profile (norm =0)	Confirmatory plasma amino acids in µmol/L	Confirmatory urine FIGLU in (norm=undetectable)	Confirmatory hydantoin-5-propionic acid on urine organic acids in mg/g Cr	Confirmatory relate level elevated?
17 (MI)	2.38	+	0.48	increased	no significant abnormalities	large amount	not done	+
18 (MI)	1.84	+	normal	increased	no significant abnormalities	71.8	not doene	+
MEAN:	**2.1**		**1.5**	**1.9**		**108.1**	**86.5**	**15/15 elevated**

Table 3

Genotype and outcomes

Subject	Allele 1	Allele 2	Age last outcomes data available (months)	Most recent hemoglobin (g/dL)	Development at last evaluation
1	c.990dupG (p.P331Afs*2)	c.990dupG (p.P331Afs*2)	11	normal	normal
2	c.990dupG (p.P331Afs*2)	c.990dupG (p.P331Afs*2)	48	normal	autism, speech delay, requires individualized education plan
3	c.990dupG (p.P331Afs*2)	c.99GdupG (p.P331Afs*2)	27	10.3 (norm 10.5-13.5)	normal
4	C.1366dupG (p.E456Gfs*56)	c.236A>C(p.Q79P)	3	not done	normal
5	c.990dupG (p.P331Afs*2)	c.1366dupG (p.E456Gfs*56)	20	not done	resolved mild speech delay
6	c. 1607T>A (p.L536X)	c. 1607T>A [p.L5 3 6X)	26	10.9 (norm 11.5-13.5)	normal
7	not done	not done	62	12.6 (norm 13.5-19.5)	normal
8	c.99DdupG (p.P331Afs*2)	c.763C>T (p.R255X)	102	normal	mild cognitive delay, requires individualized education plan
9	c.223A>C(p.M75L)	c.99GdupG (p.P331Afs*2)	36	normal	normal
10	not done	not done	101	normal	normal
11	not done	not done	98	9.2 (norm 10.4-14.5)	normal
12	c.990dupG (p.P331Afs*2)	c.514G>T(p.G172W)	48	normal	normal
13	not done	not done	85	normal	normal
14	not done	not done	48	normal	mild articulation concerns
15	not done	not done	60	normal	normal
16	not done	not done	98	normal	normal
17	c.1607 T>A (p.L536X)	c.1607 T>A (p.L536X)	69	normal	speech and social skills delay, requires individualized education plan
18	c.451A>T (p.K151X)	c.319C>T (p.C107R)	66	normal	normal
mean			**59**	**4/16 with mild anemia**	**2/13 with mild spech concerns 3/13 require educational accomodations**

Table 4

Comparison to previously reported cases

		Previous Cases*	This Report
Testing Indication	ID	6/14	
	speech delay	3/14	
	tumor	3/14	
	affected sibling	1/14	
	NBS	1/14	18/18
Cognitive Delays	severe	5/14	0/18
	mild to moderate	1/14	3/18
	speech delay only	4/14	2/18
	none	4/14	13/18
Anemia	anemia	3/14	3/18
	none	10/14	13/18
	unknown	1/4	2/18

* Previous cases from references (Arakawa et al. 1965; Arakawa et al. 1968; Perry et al. 1975; Niederwieser et al. 1976; Beck et al. 1981; van Gennip et al. 1994)